Taxonomic Identification
R-18-0001
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(Producing Algae for Coproducts and Energy Consortium)

Purpose of genetic modification

The purpose of this genetic modification and subsequent field experiment is to: 1) evaluate the translatability of GM phenotypes from a lab to an outdoor setting, and 2) to compare the resistance of the GM strains to biotic (bacterial) and abiotic (diurnal temperature and solar insolation) in the subject microorganisms versus the recipient (wild type) strain.

Identification of Parent used in R-18-0001

USEPA has received a designated non-CBI TERA application similar to one received last year, case [redacted], for the same species *Chlorella sorokiniana* but for a different strain, this time *C. sorokiniana* PACE_Cs1412_SNRK2 (from now on known as Cs1412_SNRK2). The submitter identifies the parental organism as *Chlorella sorokiniana* DOE1412. This strain was isolated from the field by Juergen Polle in 2013 (UTEX website, accessed 09/2018) and deposited to the CUNY collection. Subsequently, the National Alliance for Advanced Biofuels and Bio-Products (NAABB) consortium, after a screening process has made 30 of their best performing strains, including DOE1412, made this strain available to the public through UTEX. These UTEX strains have been well characterized by DOE for lipid production and growth kinetics. UTEX and DOE, describe the strain as a high temperature freshwater strain (cold-sensitive) with a maximum growth temperature of 42 deg C. The strain is also referenced as DOE1412, NAABB 1412 and NAABB 2412.

Krienitz et al. (2015) indicate that *Chlorella* species have been widely misclassified when using traditional morphological classification schemes. This problem has been addressed with the introduction of chemotaxonomy to *Chlorella* and other taxa. Now, our understanding of the taxonomy of *Chlorella* has changed radically. Based on integrative or polyphasic taxonomy a new system has been established which differs completely from the traditional artificial system of *Chlorella* and its relatives based on morphology. *Chlorella* sensu stricto are now placed in the class Trebouxiophyceae. Luo et al. (2010) state that in the traditional context and also according to the first studies that included molecular and phylogenetic investigations, members of the genus *Chlorella* represent the archetype of a green spherical cell propagating purely by autosporulation (Huss et al. 1999). These cells do not have mucilagineous envelopes or other cell wall ornamentation. They contain a single chloroplast with a pyrenoid. The pyrenoid is covered by a starch envelope and traversed by thylakoid membranes. Krienitz et al. (2004) had suggested that only three 'true' spherical species belong to this genus: *Chlorella vulgaris, C. lobophora,* and *C. sorokiniana*. In more recent years, however the use of additional

molecular and polyphasic taxonomic methods has gradually increased the number of authentic *Chlorella* species (Luo et al., 2010; Bock et al., 2011; Krienitz et al., 2012, Zuo et al., 2016). This number had reached 14 with the inclusion of several former *Dictyosphaerium* strains (Bock et al., 2011), with suggestions of others possible (Zuo et al., 2016). C*. vulgaris* is the type species of this genus (Shihira et al., 1965) and *C. sorokiniana* has retained authentic species status throughout these various taxonomic revisions.

The submitters provided the following information to support the assignment of DOE1412 to *C. sorokiniana*:

The recipient strain for this project will be *C. sorokiniana* DOE1412. This organism can be identified by running a whole cell approach to PCR with the specific primers developed for allowing discrimination from other *Chlorella sp*., even specific strains within species. The *C. sorokiniana* 1412 specific primers are a) FWD 5' GCGAAGAAGAAAATGTAAACTTATTAG 3' and b) Rev 5' CCATTCCAGTAATTGCTAAATCA 3'.

Of note and with respect to Figure 1 of the TERA application, Rosenberg et al. (2014) used strains of *C. variabilis* that cluster within the group that some suggest are the true *Chlorella.* The tree provided shows that *C. sorokiniana* clusters separately from *C. vulgaris* and *C. variabilis* strains, its closest neighbors in that study.

Intergeneric Donor

The intergeneric gene used to develop the strains in this TERA, the *SNRK2* gene was derived from a *Picochlorum soloecismus* strain, a genus of green algae in the class Trebouxiophyceae.

Picochlorum soloecismus is a halotolerant, fast-growing and moderate lipid producing microalga that has been evaluated as a renewable feedstock for biofuel production by the DOE (Gonzalez-Esquer et al., 2018).

The sucrose non-fermenting (SNF) related kinase gene, *SNRK2*, is part of the serine/threonine kinases (Kertesz et al., 2002) and plays a key role in sugar metabolism in plant and animal kingdoms and controls multiple growth and metabolic processes. The submitter is utilizing *SNRK2* gene to improve photosynthetic efficiency and biomass in the recipient organism The gene was synthesized in its native state (only the coding regions) without codon optimization and cloned into the PACE *Chlorella* plasmid vector. The regulatory elements used to express the SNRK gene are the *psaD* (a photosynthesis-related gene) and *actin* promoters and terminators, both of which are endogenous to the recipient microorganism.

References

1. Bock, C., Krienitz, L., & Pröschold, T. (2011). Taxonomic reassessment of the genus *Chlorella* (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. Fottea, 11(2), 293-312.

2. Gonzalez-Esquer, C. R., Twary, S. N., Hovde, B. T., & Starkenburg, S. R. (2018). Nuclear, Chloroplast, and Mitochondrial Genome Sequences of the Prospective Microalgal Biofuel Strain *Picochlorum soloecismus*. Genome Announcements, 6(4). doi:10.1128/genomeA.01498-17
3. Huss, V. A., Frank, C., Hartmann, E. C., Hirmer, M., Kloboucek, A., Seidel, B. M., Wenzeler, P., Kessler, E. (1999). Biochemical taxonomy and molecular phylogeny of the genus *Chlorella* sensu lato (Chlorophyta). Journal of Phycology, 35(3), 587-598.
4. Kertesz, N., Samson, J., Debacker, C., Wu, H., & Labastie, M. C. (2002). Cloning and characterization of human and mouse SNRK sucrose non-fermenting protein (SNF-1)-related kinases. Gene, 294(1-2), 13-24.
5. Krienitz, L., & Bock, C. (2012). Present state of the systematics of planktonic coccoid green algae of inland waters. Hydrobiologia, 698(1), 295-326.
6. Krienitz, L., Hegewald, E. H., Hepperle, D., Huss, V. A., Rohr, T., & Wolf, M. (2004). Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov. (Chlorophyta, Trebouxiophyceae). Phycologia, 43(5), 529-542.
7. Krienitz, L., Huss, V. A., & Bock, C. (2015). *Chlorella*: 125 years of the green survivalist. Trends in plant science, 20(2), 67-69.
8. Luo, W., Pröschold, T., Bock, C., & Krienitz, L. (2010). Generic concept in *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). Plant biology, 12(3), 545-553.
9. Rosenberg, J. N., Kobayashi, N., Barnes, A., Noel, E. A., Betenbaugh, M. J., & Oyler, G. A. (2014). Comparative analyses of three *Chlorella* species in response to light and sugar reveal distinctive lipid accumulation patterns in the microalga *C. sorokiniana*. PloS one, 9(4), e92460.
10. Shihira, I., & Krauss, R. W. (1965). *Chlorella*: physiology and taxonomy of forty-one isolates: University of Maryland.
11. UTEX Website. UTEX B 3016 strain. https://utex.org/products/utex-b-3016